Global Change Biology

Global Change Biology (2013) **19**, 282–290, doi: 10.1111/gcb.12011

Sensitivity of coral calcification to ocean acidification: a meta-analysis

NEIL C. S. CHAN* and SEAN R. CONNOLLY*

**School of Marine and Tropical Biology, James Cook University, Townsville, Qld 4811, Australia*

Abstract

To date, meta-analyses of effects of acidification have focused on the overall strength of evidence for statistically significant responses; however, to anticipate likely consequences of ocean acidification, quantitative estimates of the magnitude of likely responses are also needed. Herein, we use random effects meta-analysis to produce a systematically integrated measure of the distribution of magnitudes of the response of coral calcification to decreasing Ω_{Arag}. We also tested whether methodological and biological factors that have been hypothesized to drive variation in response magnitude explain a significant proportion of the among-study variation. We found that the overall mean response of coral calcification is ~15% per unit decrease in Ω_{Arag} over the range $2 < \Omega_{Arag} < 4$. Among-study variation is large (standard deviation of 8% per unit decrease in Ω_{Arag}). Neither differences in carbonate chemistry manipulation method, study duration, irradiance level, nor study species growth rate explained a significant proportion of the among-study variation. However, studies employing buoyant weighting found significantly smaller decreases in calcification per unit Ω_{Arag} (~10%), compared with studies using the alkalinity anomaly technique (~25%). These differences may be due to the greater tendency for the former to integrate over light and dark calcification. If the existing body of experimental work is indeed representative of likely responses of corals in nature, our results imply that, under business as usual conditions, declines in coral calcification by end-of-century will be ~22%, on average, or ~15% if only studies integrating light and dark calcification are considered. These values are near the low end of published projections, but support the emerging view that variability due to local environmental conditions and species composition is likely to be substantial.

Keywords: aragonite saturation state, carbonate chemistry, climate change, CO_2, coral calcification, coral reefs, meta-analysis, pH

Received 21 May 2012; revised version received 6 August 2012 and accepted 8 August 2012

Introduction

Crucial to the capacity of coral reefs to provide various ecological and economic goods and services is corals' ability to form three-dimensional skeletal structures through the process of calcification (Moberg & Folke, 1999). One ongoing environmental change that has potential negative impacts on coral calcification is ocean acidification (Doney *et al*., 2009). Ocean acidification refers to the lowering of the pH of the oceans due to rising atmospheric carbon dioxide (CO_2), which is caused by fossil fuel burning (Sabine *et al*., 2004). Under the Intergovernmental Panel on Climate Change's 'business as usual' (IS92a) scenario, increases in atmospheric CO_2 will cause oceanic pH to decrease by 0.77 units by the year 2300 (Caldeira & Wickett, 2003), altering the current distribution of dissolved inorganic carbon (DIC) ion species in seawater, causing a reduction in carbonate (CO_3^{2-}) and the saturation state of aragonite (Ω_{Arag}):

$$\Omega_{Arag} = \frac{[Ca^{2+}][CO_3^{2-}]}{k_{sp}}$$

where $[Ca^{2+}]$ and $[CO_3^{2-}]$ are concentrations of calcium and carbonate, respectively, and K_{sp} is the solubility constant for a particular mineral phase of $CaCO_3$ (Stumm & Morgan, 1981). The changes in Ω_{Arag} are of particular relevance for coral calcification because precipitation of aragonite, the building block of coral skeleton, is increasingly facilitated as Ω_{Arag} increases above one. Coral reefs in the modern ocean are restricted to regions where oceanic Ω_{Arag} exceeds ~3.3 (Kleypas *et al*., 1999), and coral calcification rate has been found to vary positively with Ω_{Arag} in experimental studies (Schneider & Erez, 2006).

Although there is broad agreement that ocean acidification will lead to decreased coral calcification, considerable uncertainty remains about the likely magnitude of the effect (i.e. the amount by which calcification will decline in response to a given decrease in Ω_{Arag}), and about the factors that may drive geographic and interspecific variation in the calcification response. The most recent IPCC report projected a 20%–60%

Correspondence: Neil Chan, tel. + 61 431 570 024, fax + 61 747 815 511, e-mail: neil.chan@my.jcu.edu.au

reduction in coral calcification with doubling of atmospheric pCO_2 (roughly 34% decline in Ω_{Arag}), and stated that by 2070, many reefs could reach critical Ω_{Arag} (Parry *et al.*, 2007). A more recent projection suggests a response toward the upper end of this range, with many reefs experiencing net dissolution by mid-century (Silverman *et al.*, 2009). Some reviews report an average 30% decline in calcification in response to doubling of pCO_2 (Kleypas *et al.*, 2006), whereas ,however, other reviews argue that it remains still unclear to what extent ocean acidification will influence calcification and call for more research (Atkinson & Cuet, 2008). Existing reviews that have graphically compared calcification response with ocean acidification from multiple studies have all noted both an overall tendency for calcification to decline as Ω_{Arag} declines, and a high degree of variability in apparent rates of decline. Several hypotheses have been proposed to explain this variability (e.g., Langdon & Atkinson, 2005; Pandolfi *et al.*, 2011; McCulloch *et al.*, 2012). For instance, differences in carbonate chemistry manipulation method, duration of study, irradiance levels, coral energetic status, and study species growth rate, have all been proposed as a possible cause of variation in results, but experimental studies explicitly investigating these hypotheses have yielded mixed results (Marubini *et al.*, 2001, 2003; Cohen & Holcomb, 2009; Schulz et al., 2009; Krief *et al.*, 2010; Rodolfo-Metalpa *et al.*, 2010). Thus, understanding variability in the calcification response remains an active research area (Pandolfi *et al.*, 2011).

The number of experimental studies seeking to estimate the sensitivity of calcification to Ω_{Arag} has increased dramatically in recent years, but the magnitude of the calcification response estimated in these studies has varied enormously, from an increase in 25% to a decrease in 66%, per unit decrease in Ω_{Arag}. For this body of work to inform our understanding of the likely response of calcification to ocean acidification, a quantitative approach to synthesizing the information from these studies is required. Meta-analysis is an analytical method for combining evidence from multiple studies, and for identifying the factors that explain variation between studies in measured experimental effects (Gurevitch & Hedges, 1999). To date, there have only been two meta-analyses published on the effects of ocean acidification. Hendriks *et al.* (2010) examined the survival, metabolism, calcification and growth of bivalves, coccolithophores, coral, cyanobacteria, phytoplankton, and seagrasses, whereas Kroeker *et al.* (2010) examined the survival, calcification, growth and photosynthesis (where applicable) of calcifying algae, coral, coccolithophores, molluscs, echinoderms, crustaceans, fish, fleshy algae, and sea grasses. Both studies confirmed that, overall, acidification causes a significant decrease in coral calcification. Moreover, Kroeker *et al.* (2010) considered the method of carbonate chemistry manipulation, and the duration of experiments, but found no evidence that either explained a significant proportion of the among-study variation in effect size. These two studies used 'effect size' meta-analysis. This allows an assessment of whether ocean acidification has a positive, negative, or no significant effect on a response variable, such as calcification. However, because this compares control and treatment effects without regard to the magnitude of the treatment imposed (Gurevitch & Hedges, 2001), and because the magnitude of decline in Ω_{Arag} varies dramatically among studies (e.g., from 0.8 to 2.5 in Hendricks *et al.* 2010), this approach cannot be used to quantitatively estimate the sensitivity of calcification to a given decline in Ω_{Arag}. The effect-size approach also complicates interpretation of tests for differences between groups of studies, as statistical power may be impaired by differences between studies in the magnitude of decline in Ω_{Arag} imposed in experimental treatments.

Herein, we quantitatively synthesize the available experimental evidence to produce an overall estimate of the sensitivity of calcification to changes in Ω_{Arag}, and to determine how well among-study variation in the calcification response can be explained by biological and methodological differences between studies. Specifically, we use a random effects meta-analysis of regression slopes to produce a combined mean slope for calcification against Ω_{Arag}. We test carbonate chemistry manipulation methods, calcification measurement methods, study duration, irradiance level and species growth rate, as possible drivers of variation in responses between studies. We also assess the possibility of publication bias.

Materials and methods

Data selection

Our meta-analysis included 25 published estimates of the relationship between calcification and Ω_{Arag}, (Table S1). This collection of studies was compiled by searching the biological literature for studies that reported the effects of altered seawater chemistry on coral calcification. Literature searches were conducted using the ISI Web of Science database for the relevant keywords: coral calcification AND (ocean acidification OR increased CO_2 OR carbonate chemistry OR aragonite saturation state). We also searched the literature cited of all studies identified in that search. Studies were collected for analysis until 30 June 2011.

We collected studies that reported calcification responses to decreases in Ω_{Arag} among populations of a single species as well as responses in multiple species assemblages. We then restricted our dataset to those studies reporting pH and total alkalinity (TA) values for the given manipulations. This was carried out to obtain a consistent measure of Ω_{Arag} because there are four different pH scales (total, free, NBS, and seawater scale) and a number of different carbonate system calculation programs (e.g., Seacarb and CO2SYS) used in the literature. As regression-based meta-analysis assumes a linear relationship between the explanatory and response variable, we took several steps to ensure that nonlinearity in the relationship between calcification and Ω_{Arag} did not bias the estimates in our study. First, we restricted our dataset by excluding studies, which had a minimum Ω_{Arag} larger than three or a maximum Ω_{Arag} smaller than two. This allowed us to focus on studies that encompassed a similar range of Ω_{Arag} values (Fig. S1), and where the calcification response was likely to be approximately linear (Anthony *et al.*, 2011). It also focused our analysis on the range of values within the included studies most relevant to likely changes in tropical regions over the next century (a pH reduction from ~8.05 to ~7.8 and Ω_{Arag} reduction from ~3.5 to ~2). Secondly, we checked to confirm that there was no evidence of nonlinearity by plotting standardized residuals against standardized Ω_{Arag}: a decelerating response would produce systematically positive residuals in the middle of the range of Ω_{Arag}, whereas approximate linearity would produce unbiased residuals. Finally, we considered only those studies examining calcification during the day or across multiple days (i.e. excluding studies of dark calcification only), because previous studies have shown significant differences in sensitivity of day and night calcification to Ω_{Arag} (e.g., Anthony *et al.*, 2011).

Many studies included experimental factors in addition to Ω_{Arag} (e.g., temperature), or more than one study species. If studies tested multiple study species, these were included as separate experiments (e.g., *Acropora intermedia* and *Porites lobata* in Anthony *et al.*, 2011). However, following previous meta-analytical approaches (e.g., Kroeker *et al.* 2010), only the experiment with other factors set to ambient were included in the meta-analysis.

Data extraction and preparation

We recorded all information about the study (pH, TA, temperature, and salinity), the organism (species and growth rate) as well as methodological factors (duration of experiment, method of carbonate chemistry manipulation, and method of calcification measurement). Data were extracted from the primary literature using GraphClick (v3.0; Arizona Software, Neuchatel, Switzerland). pH, TA, temperature, and salinity were then entered into the program Seacarb (Lavigne & Gattuso 2011) in R (R Development CoreTeam, 2011) to calculate other carbonate chemistry parameters (Ω_{Arag} and DIC). Due to the many different ways that calcification is measured and reported in the literature, a way of standardizing the sensitivity of calcification to declining Ω_{Arag} was necessary so that results from various studies could be combined. Thus, we chose to standardize each study's calcification to be a percentage of a calcification at a selected baseline Ω_{Arag} level (hereafter termed baseline calcification). Previous studies have used a projected Ω_{Arag} and calcification that was outside of the experimental range as a baseline (e.g., preindustrial Ω_{Arag}: Langdon & Atkinson, 2005). However, this requires extrapolating the calcification-Ω_{Arag} response well beyond the range of the data, which can be biased even if nonlinearity in the relationship between calcification and Ω_{Arag} is relatively modest. Therefore, for our baseline Ω_{Arag}, we first calculated ambient Ω_{Arag} for each study (in Seacarb using study-specific temperature and salinity, and pCO$_2$ levels of 380). TA of seawater changes conservatively and is typically around 2300, hence, we used this value in our calculations (Kleypas & Langdon (2006). We then took the median of the ambient Ω_{Arag} values, which was 3.517, to be our baseline.

For each study, the slope (of calcification against Ω_{Arag}), its associated standard error, was estimated differently depending on whether studies reported all data points or only mean calcification and standard error at particular levels of Ω_{Arag}. For studies that reported all data points, we fit a linear regression model to the calcification vs. Ω_{Arag} data, using least squares regression (the lm() function in R) (R Development CoreTeam, 2011). Calcification was then rescaled so that predicted calcification at baseline Ω_{Arag} was 100%, and the value of the slope and its associated standard error recomputed on this normalized scale. For studies that only reported mean calcification and standard error, we used a Monte-Carlo routine to estimate the standard error of the regression slope. Specifically, using the sample size n and the standard error of calcification for each treatment, we calculated the within-treatment standard deviation of calcification. We then drew n calcification values at random for each treatment, using the appropriate mean and standard deviation, and fit a linear regression model to the Monte-Carlo sample, and noted the estimated slope, and then recalculated the slope with predicted calcification normalized to 100% at baseline Ω_{Arag}. We repeated this procedure 1000 times to obtain a bootstrap distribution of regression slopes. The standard deviation of this distribution is an estimate of the standard error of the regression slope for that study (Efron & Tibshirani, 1993).

Data analysis

There are two common meta-analysis approaches, fixed effects and random effects meta-analysis. Fixed effects meta-analysis assumes that all included studies share a common effect size (i.e. the true effect is the same for all studies), with the observed effects distributed about the common effect with a variance among studies that depends only on sampling effects (Borenstein *et al.*, 2009). In contrast, random effects meta-analysis assumes that true effect sizes exhibit random variation among studies (i.e. the 'combined effect' represents the mean of a distribution of 'true' study-specific effects), and variance among studies therefore consists of a combination of the variance of true effect sizes among studies, and sampling effects (which cause the measured effect in any one study to differ from its study-specific 'true' value) (Borenstein *et al.*, 2009). Our experiments varied widely in methodology and biological factors (such as different study species and duration of study); hence, we considered random effect meta-analysis to be most appropriate for this study. Specifically, we used the random effects procedure for combining regression slopes from Borenstein *et al.* (2009) (Appendix A).

To quantify the variability between studies, we calculated the I^2 statistic, which is the ratio of excess dispersion to total dispersion, using the procedure from Borenstein *et al.* (2009) (Appendix A). To examine the variation in the sensitivity of coral calcification to Ω_{Arag}, studies were separated to test for differences between *a priori* defined subgroups (see Table S1 for information on groupings). Specifically, we compared studies using different carbonate chemistry manipulation methods, because the two most commonly used approaches acid addition and CO_2 bubbling, decrease alkalinity at constant DIC, and increase DIC at constant alkalinity, respectively (Langdon & Atkinson, 2005; Gattuso & Lavigne, 2009; Rodolfo-Metalpa *et al.*, 2010). We also compared calcification measurement method (alkalinity anomaly technique vs. buoyant weighting), as most studies using the alkalinity anomaly technique measure calcification over a couple of hours (and thus measure only light calcification), whereas buoyant weighting studies integrate over both light and dark calcification. Studies measuring light and dark calcification separately have shown them to have different sensitivities to Ω_{Arag} (Leclercq *et al.*, 2000; Ohde & Hossain, 2004; Anthony *et al.*, 2011), suggesting that calcification measurement method could lead to differences in results. Finally, we tested for differences based on whether study species were fast or slow-growing, because it has been hypothesized that fast-growing corals might exhibit larger decreases in calcification due to their increased demand for carbonate (Rodolfo-Metalpa *et al.*, 2010) or an increased need to dissipate hydrogen ions (Jokiel, 2011). Growth rate classifications were based on literature values for the study species, or values for the most-closely related species with similar growth forms that we could find. The fast-growing category had linear extension rates >3 cm yr^{-1} and included the branching Acropora and Stylophora and the plating Turbinaria, whereas slow-growing species had estimated linear extension rates <2 cm yr^{-1} and included all other genera. To test for differences among these *a priori* defined groups, we performed separate random effects meta-analyses for each hypothesis and compared effects between subgroups using a *Z*-test (Borenstein *et al.*, 2009: summarized in Appendix A).

Meta-regression is a tool used in meta-analysis to examine the impact of among-study variation in the value of continuously varying independent variables on study effect size using regression-based techniques. Meta-regressions were carried out to test for an effect of study duration and irradiance level on sensitivity of calcification to decreasing Ω_{Arag}, following the procedure for weighted regression that incorporates residual heterogeneity by including an additive between-study variance component (model 3a in Thompson & Sharp, 1999; summarized in Appendix A). As both study duration and irradiance level varied by orders of magnitude across studies they were log-transformed to obtain a more even spread in the independent variable of the regression (transformations did not affect the statistical significance of the effects). The effect of study duration was tested because it has been suggested that, due to the possibility of coral acclimation, studies conducted over weeks or months are likely to show less sensitivity of calcification to decreasing Ω_{Arag}, compared with studies lasting less than a day (Langdon & Atkinson, 2005; Krief *et al.*, 2010; Pandolfi *et al.*, 2011). The effect of irradiance level was tested because light is known to be an environmental parameter that has a strong effect on calcification (Barnes, 1982) and previous study has found that the reduction in calcification by decreased Ω_{Arag} was greater in corals in high light than in corals in low light (Marubini *et al.*, 2001).

Publication bias

Publication bias occurs whenever the strength or direction of the results of published studies differ from those of unpublished studies (Moller & Jennions, 2001). Two independent methods were used to investigate whether publication bias occurs in the ocean acidification literature. The first method was visual inspection of a 'funnel graph' of sample size against estimated slope (Moller & Jennions, 2001). If slopes derive from a random sample of studies using similar research methods, a plot of sample size against estimated slope should reveal a funnel centered on the weighted mean slope, with larger variation in values at small sample sizes and decreasing variance with increasing sample size (Jennions *et al.*, 2001; Moller & Jennions, 2001). We also calculated the 'fail-safe number', *X*, for the dataset: this is an estimate of the number of future studies needed to change a significant effect to a non-significant one (Moller & Jennions, 2001). Rosenthal (1991) suggests that a fail-safe number at least five times larger than the number of studies plus ten indicates that publication bias is unlikely to alter conclusions about statistical significance from the meta-analysis (see Appendix A for details).

Results

We found 30 studies that quantified the calcification responses of corals to ocean acidification and of those, 25 studies met our criteria (Table S1). Meta-analysis of these data revealed a significant negative effect on calcification, with an average 15% decline in calcification per unit decline in Ω_{Arag}, and an among-study standard deviation of 8% (Fig. 1). This heterogeneity in the calcification responses was large, relative to measurement error (I^2 = 85.36), indicating that among-study variation reflected real differences in biology or methodology among studies and that a distribution of true means better reflected the data than a single fixed effect magnitude. In particular, estimated 95% confidence intervals on study-specific effects (i.e. combined slope ± 1.96 times the among-study standard deviation) ranged from 0% to 31% per unit of Ω_{Arag}. Inspection of standardized residuals vs. standardized Ω_{Arag} suggested than any nonlinearity present for the studies, we included was small, relative to the residual variation. Specifically, residuals were symmetrically

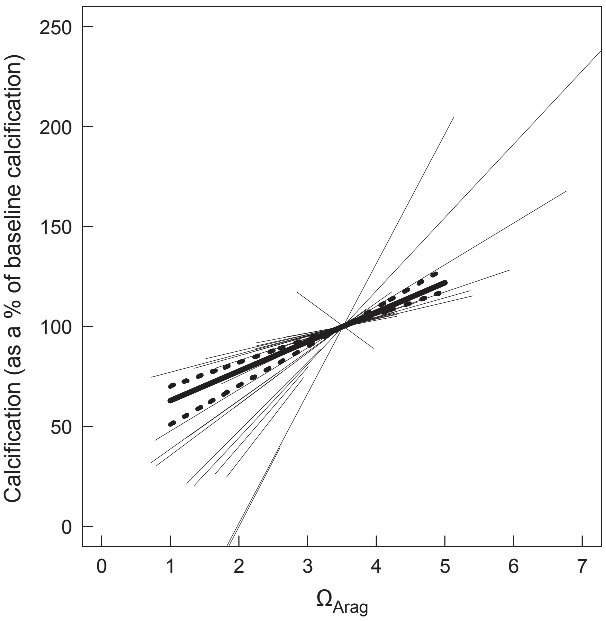

Fig. 1 Overall effects of ocean acidification on coral calcification. Calcification is denoted as a percentage decrease from baseline calcification (calcification at Ω_{Arag} of 3.517) per unit decrease in Ω_{Arag}. Thus, all lines intersect the point (Ω_{Arag} = 3.517, calcification = 100%). The thin black lines show the calcification responses for individual studies. The endpoints of these lines indicate the range of Ω_{Arag} values spanned in each study. The thick black line represents the combined (mean) calcification response across all studies, and the dashed lines represent upper and lower 95% confidence intervals for this combined response (that is, they represent the uncertainty around the mean response, not the overall among-study variability).

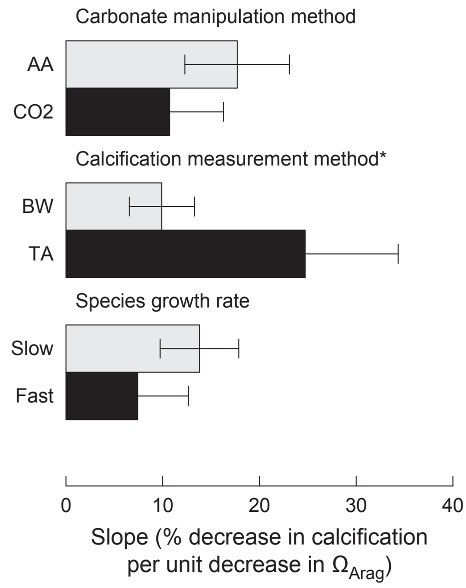

Fig. 2 Methodological and biological variation in effects of ocean acidification on coral calcification. Bars indicate the mean decrease in calcification per unit decrease in Ω_{Arag} for each subset of studies. Whiskers indicate 95% confidence intervals on the mean response. AA, acid addition studies; CO_2, CO_2 bubbling studies; BW, buoyant weighting studies; TA, alkalinity anomaly technique studies; Slow, studies using slow-growing species; Fast, studies using fast-growing species. The slopes are significantly different only using calcification measurement method (indicated with an asterisk).

distributed around zero, exhibiting no evidence of linear or curvilinear trends (Fig. S2), indicating that linear regression slopes provide an adequate approximation for the calcification response for the studies in our analysis.

Experiments that manipulated carbonate chemistry using acid addition (varying TA at a constant DIC) and those that modified pH using CO_2 bubbling (increasing DIC at a constant TA) did not differ significantly ($Z = 1.76$, $P = 0.08$; Fig. 2). In contrast, studies that measured calcification using the total alkalinity method showing a 25% decline in calcification per unit decline in Ω_{Arag}, which was significantly larger than the 10% decline shown in studies that measured calcification using the buoyant weighting method ($Z = 2.85$, $P = 0.004$; Fig. 2). The effects of Ω_{Arag} on calcification also did not differ significantly among experiments using fast vs. slow-growing coral taxa ($Z = 1.88$, $P = 0.06$; Fig. 2). Between-study variability in sensitivity of calcification to Ω_{Arag} was not significantly explained by either study duration ($t = 0.146$, $P = 0.89$; Fig. 3a) or irradiance level ($t = -0.773$, $P = 0.45$; Fig. 3b).

Publication bias

The fail-safe number was over an order of magnitude larger than five times the number of studies plus ten ($X = 2681 \gg 135$), indicating that the overall negative effect of decreasing Ω_{Arag} on calcification is very robust to any publication bias that may be present. However, inspection of the funnel plot does suggest a bias toward publication of studies that find negative effects of decreasing Ω_{Arag} (i.e. a positive slope of calcification vs. Ω_{Arag}: Fig. 4). If slopes derive from a random sampling of studies using similar research methods, a plot of sample size against slope should reveal values distributed within a funnel (solid lines in Fig. 4) symmetrically around the weighted mean slope (dashed line in Fig. 4), with larger variation in values at small sample sizes (studies with small sample size are less precise), and a decreasing variance with increasing sample size. In our study, while there were roughly the same number of studies on both sides of the weighted mean slope, the distribution of estimated slopes was highly

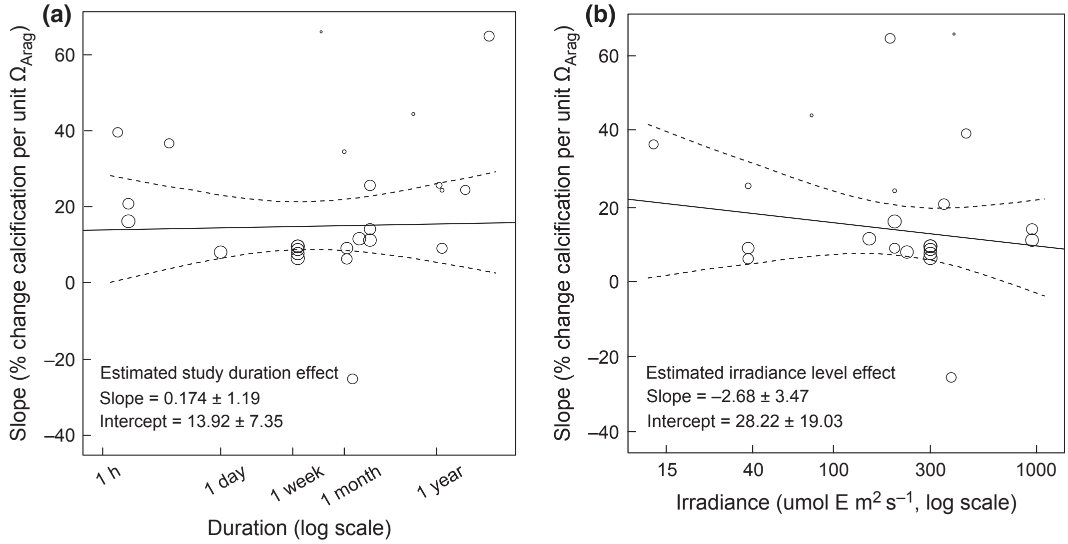

Fig. 3 Slope (percentage change in calcification per unit Ω_{Arag}) against (a) duration of experiment on the log scale, with ticks below indicating (left to right) durations of an hour, a day, a week, a month and a year (b) irradiance level. The circles correspond to each study and have area proportional to the study's weighting (reciprocal of the variance of the slope estimate). The line is obtained by weighted least squares regression using a maximum likelihood estimate of the residual heterogeneity. Note that the 'slope' and 'intercept' values reported in the figure panel are estimates of the parameters (with standard errors) that describe how the slope of the calcification–Ω_{Arag} relationship changes as a function of (a) study duration and (b) irradiance.

asymmetric: values above the weighted mean slope were broadly distributed within the funnel, whereas values below the weighted mean slope were, with only one exception, concentrated above zero (dotted line in Fig. 4), very close to the weighted mean slope (Fig. 4).

Discussion

Our random effects meta-analysis found that coral calcification declines by ~15% on average per unit decrease in Ω_{Arag}, but with considerable among-study variability. If existing experimental studies are indeed representative of the likely response to acidification in nature, this finding implies that, on average, calcification will decline by ~22% by 2100, under a 'business as usual' emissions scenario. Specifically, assuming pCO_2 doubles from 400 to 800 ppm and Ω_{Arag} decreases from 3.5 to 2, the consensus from reviews is for a 20%–60% reduction in coral calcification by the end of the 21st century (Langdon & Atkinson, 2005; Kleypas *et al.*, 2006; Parry *et al.* 2007). Our estimate is within, but toward the low end of, the range of likely responses to acidification that have been proposed in earlier study. However, the large between-study variability indicates that, whereas some corals' responses are likely to fall below the range of estimates from previous study, others will be toward the middle or potentially upper end of the range. The decreases suggested by our analysis are not trivial, but they do suggest a consensus distribution of responses to acidification from experimental studies that may be less severe than has been suggested by some recent reviews and models (e.g., Hoegh-Guldberg *et al.*, 2007; Silverman *et al.*, 2009).

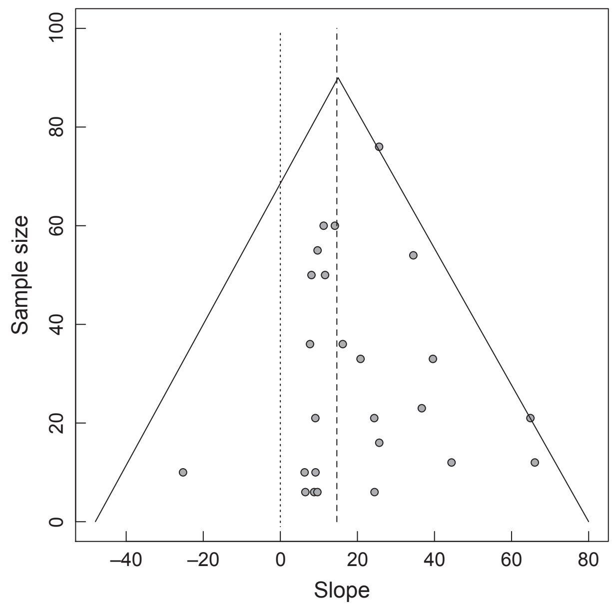

Fig. 4 Funnel plot depicting the relationship between sample size and slope. The points are individual studies, each with their own sample size and slope. The dotted line is a slope of zero (decreasing Ω_{Arag} has no effect on calcification). The dashed line is the observed weighted mean slope. The solid lines are an illustration of a funnel that should be formed by the points if there is no publication bias.

Our results also reveal that studies measuring calcification via the alkalinity anomaly (TA) method found significantly larger decreases in calcification than studies using buoyant weighing. This would seem to contradict recent experiments that show no difference in decreases between calcification measured by TA or by buoyant weight, when all other factors are held constant (Holcomb *et al.* 2010). One possible explanation of this is that buoyant weighting studies, of necessity, estimate calcification over relatively long time scales (weeks to years). Consequently, they implicitly integrate over both light and dark calcification. In contrast, TA measurements can be made over very short intervals, even when studies themselves are conducted over a long period. Typically, these measurements are made during the day and thus include only effects of acidification on light calcification. There is some evidence that the decrease in dark calcification with decreasing Ω_{Arag} is less pronounced than that of light calcification (Leclercq *et al.*, 2000; Anthony *et al.*, 2011). If this is a common phenomenon, then the average decrease across light and dark calcification measured in buoyant weighting studies would be less than the decrease in light calcification alone measured in TA studies. Consistent with this explanation, Holcomb *et al.* (2010), who found no difference between the two methods, were unusual in carrying out their TA analysis over a 2 day period, thereby incorporating both light and dark calcification in both TA and buoyant weighting measurements. Our interpretation of this discrepancy between buoyant weighting and alkalinity anomaly studies warrants further testing. If correct, it would indicate that the calcification response is likely to be somewhat weaker than our headline result suggests: a decline of 10%, on average, per unit decrease in Ω_{Arag}, with 95% intervals on the among-study variation in 5.5–14.5%.

In contrast to the calcification measurement method, we found no significant difference between mean slopes for CO_2 bubbling and acid addition methods, consistent with previous findings from effect-size meta-analysis (Kroeker *et al.* 2010), and with reviews of methodology, which indicate that differences in speciation of the carbonate system, for moderate pCO_2 levels, is small enough so as not to lead to differences in calcification (Cohen *et al.*, 2009; Schulz *et al.* 2009; Gattuso *et al.*, 2010; de Putron *et al.*, 2011). Although fast-growing corals have been hypothesized to be more sensitive to acidification than slow-growing corals (Rodolfo-Metalpa *et al.*, 2010), we did not find significant differences between estimated slopes for experiments on fast vs. slow-growing corals. Similarly, although acclimation has been hypothesized to reduce the sensitivity of calcification to decreasing Ω_{Arag} (Pandolfi *et al.*, 2011), we found no evidence that such a phenomenon explains significant variation in calcification sensitivity among studies in our analysis. We also found that differences in irradiance level did not explain significant variation in calcification sensitivity among studies in our analysis. These findings do not mean that growth rate, acclimation, or light have no effect on the response of calcification to acidification, but they do indicate that these factors do not account for a statistically significant proportion of the large among-study variability in the calcification response documented to date.

The mean sensitivity that we have produced could be an overestimate if the published studies are a biased sample of those conducted. Publication bias has only been assessed once in previous studies, which reported a large fail-safe number, but conjectured nevertheless that the published literature is probably biased toward studies that find significant effects (Kroeker *et al.* 2010). We too determined the fail-safe number, which is the standard way of analyzing publication bias (Gurevitch & Hedges, 1999), and also found that the conclusion that calcification is negatively affected by deceasing Ω_{Arag} is robust, consistent with previous work. However, the large fail-safe number does not confirm necessarily the robustness of the magnitude of that negative effect, and our funnel plot suggests that publication bias may well be present: studies to the left of the mean response are concentrated near it, rather than being spread more evenly within the left half of the funnel. This result should be interpreted with caution because skewed funnel plots may also be caused by other factors, such as previous knowledge of effect sizes from pilot studies, reduced sample sizes for certain species, choice of effect measures and chance (Moller & Jennions, 2001). The large variability in experimental techniques and lack of information about the role of prior knowledge in experimental design in published studies makes it difficult to rule out these other factors.

Our finding that calcification responses, on average, are likely to fall toward the lower end of the range reported in the last IPCC report (Parry *et al.* 2007) is consistent with some recent studies that have sought to infer calcification response based on estimates of the extent to which corals increase pH at the site of calcification, relative to the surrounding seawater. Specifically, four different approaches (pH microsensors, aragonite crystal aspect ratios, live tissue imaging, and boron-isotope schematics) indicate consistently higher pH at the site of calcification compared with the surrounding seawater (Al-Horani *et al.*, 2003; Cohen *et al.*, 2009; Venn *et al.*, 2011; McCulloch *et al.*, 2012). This provides a potential explanation of why coral calcification changes less steeply with seawater Ω_{Arag}, on average, than one would predict based on abiogenic aragonite precipitation rates (e.g., Langdon & Atkinson, 2005; Silverman *et al.*, 2009). For instance, the calibration of McCulloch *et al.* (2012) implies an average decline in calcification of ~11% per unit Ω_{Arag} when Ω_{Arag} is close to the median value from the studies in our meta-analysis (obtained by normalizing the calcification rates in their Fig. 2 to calcification at $\Omega_{Arag} = 3.5$, and then numerically differentiating the curve at this value). This is similar to our overall mean slope of 15%, and virtually identical to the mean slope of 10% obtained from our meta-analysis of the buoyant weighting subset of studies. McCulloch *et al.* (2012) also found that the ability of corals to elevate calcification site Ω_{Arag} differed between species, suggesting that this varying ability to elevate calcification site Ω_{Arag} could be a possible explanation of the large among-study variance that we found.

Our findings indicate that, while that ocean acidification will have significant negative consequences for coral calcification by the end of this century, this decline will be, on average, toward the low end of the range of responses that have been suggested in the literature. Nevertheless, even a relatively small (compared with previous projections) 15% decrease in coral calcification, has the potential to materially alter the accretion/erosion balance of reefs, particularly, if climate change-induced increases in reef dissolution occur simultaneously (Langdon *et al.*, 2000; Yates & Halley, 2006), and if other reef calcifiers, such as crustose coralline algae and calcareous benthic macroalgae, are more susceptible to ocean acidification than corals (Price *et al.*, 2011; Diaz-Pulido *et al.*, 2012). Moreover, there is some evidence that prevailing Ω_{Arag} levels on shallow-water reefs may be lower or higher than nearby open-ocean values, depending on whether they are net carbon sources or sinks (Kleypas *et al.*, 2011). Thus, coral dominated reefs (which are more likely to be net CO_2 sources) may tend to have lower Ω_{Arag} levels compared to those commonly used as 'ambient' in experimental studies. If there is greater sensitivity in the calcification response at lower Ω_{Arag} values (Ries *et al.*, 2010; Anthony *et al.*, 2011; de Putron *et al.*, 2011), then corals on low-Ω_{Arag} reefs may exhibit somewhat greater sensitivity to acidification than is suggested by the experimental data. A recent review highlighted the need to better understand the magnitude of the calcification response, and the causes of its variability, in order to better inform projections of ocean acidification's likely impact on coral reefs (Pandolfi *et al.*, 2011). The present study contributes to those goals, by providing a quantitative synthesis of existing experimental study on the effects of acidification on coral calcification, and evaluating some of the potential drivers of the apparent variation in the calcification responses of corals.

Acknowledgements

We are grateful to K. Anthony, C. Ferrier-Page, M. Hoogenboom and A. Cohen for helpful discussions in the interpretation of the results, and to D. Suggett and the three anonymous reviewers for providing comments and suggestions that improved the manuscript. We also thank K. Anthony, M. McCulloch and J. Falter for providing information from figures in their recent papers. This research was supported by the Australian Research Council (DP0880544 and CE0561432) and James Cook University.

References

Al-Horani FA, Al-Moghrabi SM, De Beer D (2003) The mechanism of calcification and its relation to photosynthesis and respiration in the scleractinian coral *Galaxea fascicularis*. *Marine Biology*, **142**, 419–426.

Anthony KRN, Kleypas JA, Gattuso JP (2011) Coral reefs modify their seawater carbon chemistry – implications for impacts of ocean acidification. *Global Change Biology*, **17**, 3655–3666.

Atkinson M, Cuet P (2008) Possible effects of ocean acidification on coral reef biogeochemistry: topics for research. *Marine Ecology Progress Series*, **373**, 249–256.

Barnes DJ (1982) Light response curve for calcification in the staghorn coral *Acropora acuminata*. *Comp. Biochem. Physiol. A*, **73**, 41–45.

Borenstein M, Hedges LV, Higgins JPT, Rothstein HR (2009) *Introduction to Meta-Analysis*. John Wiley & Sons, Hoboken, NJ.

Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. *Nature*, **425**, 365.

Cohen AL, Holcomb M (2009) Why corals care about ocean acidification: Uncovering the mechanism. *Oceanography*, **22**, 118–127.

Cohen AL, McCorkle DC, De PS, Gaetani GA, Rose KA(2009) Morphological and compositional changes in the skeletons of new coral recruits reared in acidified seawater: Insights into the biomineralization response to ocean acidification. *Geochemistry Geophysics Geosystems* **10**, 118–127.

Diaz-Pulido G, Anthony KRN, Kline DI, Dove S, Hoegh-Guldberg O (2012) Interactions between ocean acidification and warming on the mortality and dissolution of coralline algae. *Journal of Phycology*, **48**, 32–39.

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other CO_2 problem. *Annual Review of Marine Science*, **1**, 169–192.

Efron B, Tibshirani RJ (1993) *An Introduction to the Bootstrap*. Chapman & Hall, New York.

Gattuso JP, Lavigne H (2009) Technical Note: Approaches and software tools to investigate the impact of ocean acidification. *Biogeosciences*, **6**, 2121–2133.

Gattuso JP, Gao K, Lee K, Rost B, Schulz KG (2010) Approaches and tools to manipulate the carbonate chemistry. In *Guide to Best Practices for Ocean Acidification Research and Data Reporting* (eds. Riebesell U, Fabry VJ, Hansson L, Gattuso), pp. 41–52. Publications Office of the European Union, Luxembourg.

Gurevitch J, Hedges LV (1999) Statistical issues in ecological meta-analyses. *Ecology*, **80**, 1142–1149.

Gurevitch J, Hedges LV (2001) Meta-analysis: combining the results of independent experiments. In *Design and Analysis of Ecological Experiments* (ed. Scheiner S), pp. 347–369. Oxford University Press, New York.

Hendriks IE, Duarte CM, Alvarez M (2010) Vulnerability of marine biodiversity to ocean acidification: A meta-analysis. *Estuarine, Coastal and Shelf Science*, **86**, 157–164.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ *et al.* (2007) Coral reefs under rapid climate change and ocean acidification. *Science*, **318**, 1739–1742.

Holcomb M, McCorkle DC, Cohen AL (2010) Long-term effects of nutrient and CO_2 enrichment on the temperate coral *Astrangia poculata* (Ellis and Solander, 1786). *Journal of Experimental Marine Biology and Ecology*, **386**, 27–33.

Jennions MD, Moller AP, Petrie M (2001) Sexually selected traits and adult survival: a meta-analysis. *The Quarterly Review of Biology*, **76**, 3–36.

Jokiel PL (2011) Ocean acidification and control of reef coral calcification by boundary layer limitation of proton flux. *Bulletin of Marine Science*, **87**, 639–657.

Kleypas JA, Langdon C (2006) Coral reefs and changing seawater chemistry. In: *Coral Reefs and Climate Change: Science and Management* (eds Phinney JT, Hoegh-Guldberg O), pp. 73–110, American Geophysical Union, Washington, DC.

Kleypas JA, McManus JW, Menex LAB (1999) Environmental limits to coral reef development: where do we draw the line? *American Zoologist*, **39**, 146–159.

Kleypas JA, Feely RA, Fabry C, Langdon C, Sabine CL, Robbins LL (2006) Impacts of Ocean Acidification on Coral Reefs and Other Marine Calcifiers: A Guide for Future Research. A report of a workshop held 18–20 April 2005, St. Petersburg, FL. Sponsored by NSF, NOAA and the U.S Geological Survey.

Kleypas JA, Anthony KRN, Gattuso JP (2011) Coral reefs modify their seawater carbon chemistry – case study from a barrier reef (Moorea, French Polynesia). *Global Change Biology*, **17**, 3667–3678.

Krief S, Hendy EJ, Fine M, Yam R, Meibom A, Foster GL, Shemesh A (2010) Physiological and isotopic responses of scleractinian corals to ocean acidification. *Geochimica et Cosmochimica Acta*, **74**, 4988–5001.

Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecology Letters*, **13**, 1419–1434.

Langdon C, Atkinson MJ(2005) Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature, irradiance and nutrient enrichment. *Journal of Geophysical Research* **110**, C09S07, doi:10.1029/2004JC002576.

Langdon C, Takahashi T, Sweeney C, Chipman D, Goddard J (2000) Effects of calcium carbonate saturation state on the calcification rate of an experimental coral reef. *Global Biogeochemical Cycles*, **14**, 639–654.

Lavigne H, Gattuso JP (2011) Seacarb: Seawater Carbonate Chemistry with R. R package version 2.4.

Leclercq N, Gattuso JP, Jaubert J (2000) CO_2 partial pressure controls the calcification rate of a coral community. *Global Change Biology*, **6**, 329–334.

Marubini F, Barnett H, Langdon C, Atkinson M (2001) Dependence of calcification on light and carbonate ion concentration for the hermatypic coral *Porites compressa*. *Marine Ecology Progress Series*, **220**, 153–162.

Marubini F, Ferrier-Pages C, Cuif JP (2003) Suppression of skeletal growth in scleractinian corals by decreasing ambient carbonate-ion concentration: a cross-family comparison. *Proceedings of the Royal Society of London B*, **270**, 179–184.

McCulloch M, Falter JL, Trotter J, Montagna P (2012) Coral resilience to ocean acidification and global warming through pH up-regulation. *Nature Climate Change*, **2**, 623–627.

Moberg F, Folke C (1999) Ecological goods and services of coral reef ecosystems. *Ecological Economics*, **29**, 215–233.

Moller AP, Jennions MD (2001) Testing and adjusting for publication bias. *Trends in Ecology & Evolution*, **16**, 580–586.

Ohde S, Hossain MMM (2004) Effects of $CaCO_3$ (aragonite) saturation state of seawater on calcification of *Porites* coral. *Geochemical Journal*, **38**, 613–621.

Pandolfi JM, Connolly SR, Marshall DJ, Cohen AL (2011) Projecting coral reef futures under global warming and ocean acidification. *Science*, **333**, 418–422.

Parry ML, Canziani OF, Palutikof JP, van dLP, Hanson CE (eds) (2007) Cross-chapter case study. In: *Climate Change 2007: Impacts, Adaptation and Vulnerability*, pp 843–868. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel of Climate Change. Cambridge University Press, Cambridge, UK.

Price NN, Hamilton SL, Tootell JS, Smith JE (2011) Species-specific consequences of ocean acidification for the calcareous tropical green algae Halimeda. *Marine Ecology Progress Series*, **440**, 67–78.

de Putron SJ, McCorkle DC, Cohen AL, Dillon AB (2011) The impact of seawater saturation state and bicarbonate ion concentration on calcification by new recruits of two Atlantic corals. *Coral Reefs*, **30**, 321–328.

R Development CoreTeam (2011) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ries JB, Cohen AL, McCorkle DC (2010) A nonlinear calcification response to CO_2-induced ocean acidification by the coral *Oculina arbuscula*. *Coral Reefs*, **29**, 661–674.

Rodolfo-Metalpa R, Martin S, Ferrier-Pages C, Gattuso JP (2010) Response of the temperate coral *Cladocora caespitosa* to mid- and long-term exposure to pCO_2 and temperature levels projected for the year 2100 AD. *Biogeosciences*, **7**, 289–300.

Rosenthal R (1991) *Meta-analytic Procedures for Social Research*. Sage, Newbury Park, CA.

Sabine CL, Feely RA, Gruber N *et al.* (2004) The oceanic sink for anthropogenic CO_2. *Science*, **205**, 367–371.

Schneider K, Erez J (2006) The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral *Acropora eurystoma*. *Limnology and Oceanography*, **51**, 1284–1293.

Schulz KG, Barcelos e RJ, Zeebe RE, Riebesell U, (2009) CO_2 pertubation experiments: similarities and differences between dissolved inorganic carbon and total alkalinity manipulations. *Biogeosciences*, **6**, 2145–2153.

Silverman J, Lazar B, Cao L, Caldeira K, Erez J (2009) Coral reefs may start dissolving when atmospheric CO_2 doubles. *Geophysical Research Letters*, **36**, 1–5.

Stumm W, Morgan JJ (1981) *Aquatic Chemistry. An Introduction Emphasizing Chemical Equilibria in Natural Water* (2nd edn). John Wiley & Sons, New York

Thompson SG, Sharp SJ (1999) Explaining heterogeneity in meta-analysis: a comparison of methods. *Statistics in Medicine*, **18**, 2693–2708.

Venn A, Tambutte E, Holcomb M, Allemand D, Tambutte S (2011) Live tissue imaging shows reef corals elevate pH under their calcifying tissue relative to seawater. *PLoS ONE*, **6**, 1–9.

Yates KK, Halley RB (2006) CO_3^{2-} concentration and pCO_2 thresholds for calcification and dissolution on the Molokai reef flat, Hawaii. *Biogeosciences Discussions*, **3**, 123–154.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix A. Procedures used in the random effects meta-analysis, testing for between group differences and the meta-regressions.

Figure S1. The range of Ω_{Arag} explored in each study included in the meta-analysis. Each line represents an individual study. (sometimes encompassing multiple experiments), and extends from the minimum Ω_{Arag} to the maximum Ω_{Arag} of the study.

Figure S2. Standardized residuals, pooled across individual experiments. Residuals from each study were standardized against that study's residual standard error, and Ω_{Arag} was scaled in each study so that maximum and minimum Ω_{Arag} for each study was 100 and 0, respectively. Each combination of color and symbol represents residuals from an individual study. The solid line is a regression line relating standardized residuals against standardized Ω_{Arag}. Note that the regression has a slope of approximately zero, and there is no evidence of a curvilinear trend in the residuals, as would be apparent if there were a qualitatively consistent pattern of nonlinearity in the calcification response over the range of Ω_{Arag} values considered.

Table S1. List of studies included in the meta-analysis, including references, species, study specimen origin, carbonate manipulation method, calcification measurement method, growth rate classification, reference for the growth rate classification (if different from the study reference), duration of study, range of Ω_{Arag} used, temperature, salinity, slope, and standard deviation.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.